

**Gram +VE Bacteria**

**Genus : Clostridium**



أ.د / جمال يونس

# genus: Clostridium

## general characters:

### I Morphology:

- ◆ stain used → Gram's stain
  - ◆ staining reaction → Gram +ve
  - ◆ shape → straight rods except *C. spiroforme* which is curved or spiral cells
  - ◆ size → medium to large
  - ◆ arrangement → single, pairs or in short chains except:
    - 1- *C. septicum* → produce long filaments (snake-like) in films prepared from peritoneal surface of liver.
    - 2- *C. novyi* → produce long filaments
  - ◆ Motility → Motile with peritrichous flagella except *C. tetani* and *C. perfringens* (non-motile)
  - ◆ Capsule → Non-capsulated except *C. perfringens*
  - ◆ form endospores:
    - 1- size → larger in diameter than that of vegetative cells causing bulging of cell.
    - 2- shape → round or oval
    - 3- position → central, subterminal or terminal
- produce a characteristic diagnostic morphological feature:
- ① Round, terminal (drum stick appearance) → *C. tetani*
  - ② oval, subterminal (spoon like shape) → *C. novyi* - *C. botulinum*
  - ③ oval, central or subterminal → *C. perfringens* (rarely sporulated)
  - ④ oval, central or subterminal (lemon shape) → *C. septicum* and *C. chauvoei*.

### II Isolation:

- ◆ O<sub>2</sub> req. → obligatory anaerobic due to lack of resp. enzymes (catalase, oxidase and peroxidase)
- ◆ opt. temp → 37 °C
- ◆ opt. pH → Neutral
- ◆ Incubation time → 1-3 days
- ◆ Media used for their growth and cultivation:
  - ① Enriched liquid media → cooked meat broth (Robertson's medium), liver-liver broth (Tarozzi medium)
  - ② Solid media → sheep or horse blood agar, reinforced clostridial agar.

### III products:

They produce exotoxins.

### Classification:

- ① acc. to biochemical activities on proteins and CHO in cooked meat medium:
  - 1- proteolytic group:  
decompose protein and turns meat particles into black colour with foul odour → e.g. *C. histolyticum*.
  - 2- saccharolytic (gas gangrene) group:  
ferment CHO in meat → pink colour with production of large amount of gases (stormy fermentation) e.g. *C. perfringens*, *C. septicum*, *C. novyi* and *C. chauvoei*.
  - 3- proteolytic and saccharolytic group:  
e.g. *C. botulinum*.
  - 4- Non-proteolytic and non-saccharolytic group: *C. tetani*

## ② acc. to pathogenicity:

### Saprophytic Clostridia

- Commonly Found in Soil, Sewage and Water in Sporulated Form
- However, pathogenic clostridia (such as *C. tetani*, *C. septicum*, *C. chauvoei* and *C. novyi*) Found in Soil as saprophytes

### pathogenic Clostridia

- Normal inhabitant in the intestinal tract of man and animals and produce the disease under certain conditions.
- divided into 2 groups acc. to mechanism of disease production:

### Invasive (gas gangrene) group

- Invade and multiply in internal organs with production of Large amount of Less potent toxins.

Such as:

*C. perfringens*  
*C. septicum*  
*C. chauvoei*  
*C. novyi*

### Non-Invasive (highly toxic) group

- have no power to invade living tissues
- their pathogenicity depend on production of highly powerful neurotoxins either in:

- ① Localized infected deep wound (*C. tetani*)
- ② Contaminated canned or salted fish (*C. botulinum*)

## ③ acc. to position of Spore and gelatin

### Liquefaction:

#### 1- Subterminal spores with gelatin hydrolysis group:

*C. botulinum*, *C. perfringens*, *C. chauvoei*, *C. septicum* and *C. novyi*

#### 2- subterminal spores without gelatin hydrolysis group:

include Saprophytic clostridia e.g. *C. butyricum*

#### 3- Terminal spores with gelatin hydrolysis group:

*C. tetani*

#### 4- Terminal spores without gelatin hydrolysis group:

include Saprophytic clostridia e.g. *C. tertium*

# Toxin production

## CL. tetani

→ 2 types of exotoxins are produced by the vegetative form and not by spores.

	<b>Tetanolysin</b> (Haemolysin)	<b>Tetanospasmin</b> (Neurotoxin)
① action	Haemolysis of RBCs	acts on nervous system causing contractions of voluntary muscles due to ↑ muscular hyperactivity.
② Lethal effect to mice	—	+
③ effect of: a- Heat b- oxygen	Labile Labile	Labile stable
④ producer strains and antigenic structure	all toxigenic and non-toxigenic strains e.g. C. tetanoides and C. tetanomorphum	all toxigenic serotypes. It has one antigenic structure i.e. it is neutralized by antitoxin of all serotypes.

## Tetanospasmin

- chemical structure → simple protein
- effect of:
  - a- acid → Labile
  - b- a. 3% Formalin → Labile and converted into toxoid (important for vaccine preparation)
- It binds irreversibly to gangliosides of nerve cells so, antitoxin is not effective when binding occurs.

## CL. botulinum

produce 8 antigenically distinct exotoxins (antitoxin of one type not neutralize the toxins of other types)

Type	A	B	C <sub>α</sub>	C <sub>β</sub>	D	E	F	G
toxin	A	B	C <sub>1</sub> (C <sub>2</sub> )	C <sub>2</sub> , D (C <sub>1</sub> )	C <sub>2</sub> , D	E	F	G

● They are the most powerful toxins in the world.

● Effect of:

a- Heat → Heat stable at 60°C/30 min.  
→ Heat Labile at 80°C/30-40 min.

b- acid → acid-stable (Not affected by gastric juice)

● Mode of action:

interfere with the mechanism of release of acetyl choline from the motor nerve ending of parasympathetic nervous system causing paralysis.

● Neurotoxins → absorbed through digestive mucosa → symptoms appear after 36-96 hrs and death occurs due to paralysis of respiratory center.

CL. perfringens (C. welchii)	C. Septicum	C. chauvoei	C. novyi								
<p>pathogenic strains of C. perfringens produce <u>12 toxic (enzymatic) factors</u> which are differentiated into 2 groups:</p> <p>① <u>Major toxins</u>: 4 types</p> <ul style="list-style-type: none"> <li>● <u><math>\alpha</math>-toxin</u> → has Lecithinase activity.</li> <li>● <u><math>\beta</math>-toxin</u> → trypsin-Labile</li> <li>● <u><math>\epsilon</math> and iota</u> → protoxins which need proteolytic enzymes (such as pepsin or trypsin) to be activated.</li> </ul> <p><u>CL. perfringens</u>:</p> <p>→ Type A → <math>\alpha</math></p> <p>→ Type B → <math>\alpha</math>, <math>\beta</math> and <math>\epsilon</math></p> <p>→ Type C → <math>\alpha</math> and <math>\beta</math></p> <p>→ Type D → <math>\alpha</math> and <math>\epsilon</math></p> <p>→ Type E → <math>\alpha</math> and iota</p> <p>② <u>Minor toxins</u>: 8 types</p> <table border="0"> <tr> <td><math>\gamma</math> (Gamma)</td> <td><math>\mu</math> (Mu)</td> </tr> <tr> <td><math>\delta</math> (Delta)</td> <td><math>\eta</math> (eta)</td> </tr> <tr> <td><math>\theta</math> (Theta)</td> <td><math>\kappa</math> (Kappa)</td> </tr> <tr> <td><math>\nu</math> (nu)</td> <td><math>\lambda</math> (Lambda)</td> </tr> </table>	$\gamma$ (Gamma)	$\mu$ (Mu)	$\delta$ (Delta)	$\eta$ (eta)	$\theta$ (Theta)	$\kappa$ (Kappa)	$\nu$ (nu)	$\lambda$ (Lambda)	<p>produce <u>8 types of toxins</u>:</p> <ol style="list-style-type: none"> <li>① <u><math>\alpha</math>-toxin</u> (Lethal and necrotizing)</li> <li>② haemolysin</li> <li>③ haemagglutinin</li> <li>④ Fibrinolysin</li> <li>⑤ desoxyribonuclease</li> <li>⑥ hyaluronidase</li> <li>⑦ Collagenase</li> <li>⑧ Neuraminidase</li> </ol> <p>→ <u><math>\alpha</math>-toxin and haemolysin are shared antigenically with that of C. chauvoei</u></p> <p>● antisera of C. septicum neutralize the pathogenicity of C. septicum as well as C. chauvoei.</p> <p>● antisera of C. chauvoei neutralize the pathogenicity of C. chauvoei only.</p>	<p>produce <u>2 types of toxins</u></p> <ol style="list-style-type: none"> <li>① Lethal</li> <li>② haemolytic Toxin</li> </ol> <p>in broth containing veal infusion and glucose but Not in ordinary broth.</p>	<p>produce <u>8 types of toxins</u></p> <p>(<math>\alpha</math>, <math>\beta</math>, <math>\gamma</math>, <math>\delta</math>, <math>\epsilon</math>, <math>\theta</math>, Zeta and Exa)</p> <p><u>C. novyi</u>:</p> <ol style="list-style-type: none"> <li>① <u>Type A</u> → <math>\alpha</math>, <math>\gamma</math>, <math>\delta</math> and <math>\epsilon</math></li> <li>② <u>Type B</u> → <math>\alpha</math>, <math>\beta</math>, Zeta and Exa</li> <li>③ <u>Type C</u> → Non-toxicogenic</li> <li>④ <u>Type D</u> → <math>\beta</math>, Theta and Exa</li> </ol>
$\gamma$ (Gamma)	$\mu$ (Mu)										
$\delta$ (Delta)	$\eta$ (eta)										
$\theta$ (Theta)	$\kappa$ (Kappa)										
$\nu$ (nu)	$\lambda$ (Lambda)										

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# pathogenicity

## C. Tetani

Tetanus (Lockjaw) disease. كزاز الفم

It arises from:

① Contamination of deep wounds or post-operations with spores of the organism.

② post-natal tetanus:

occurs in adults post-abortion or post-delivery.

③ Neonatal tetanus:

occurs in newly born animals as a result of umbilical infection.

④ Self infection:

Where spores are present naturally in the intestinal tract (faeces) of animals.

⑤ Idiopathic tetanus:

site of infection is undiscoverable.

● Tetanos spasmin is absorbed through one of the following pathways:

↓  
Motor nerve ending to spinal cord

accompanied by spasms beginning from the wounded limb to the head and neck (ascending tetanus)

↓  
through lymphatics to blood stream then directly to brain

accompanied by spasms beginning from the head and neck to the limbs (descending tetanus)

## C. botulinum

① Types A, B, E, F and G →

Botulism in man and animals

② Type C:

- Limber neck in fowls
- Alkali disease in ducks
- Lamie sickness in cattle

③ Type D:

Lamziekte (Loin disease) in cattle

## Botulism:

is an intoxication (food intoxication) and not a bacterial infection.

→ The organism does not multiply in the living tissue but it multiplies in conserved food or decaying matter.

## C. perfringens (C. welchii)

Type	diseases	Route of infection
A	① Malignant oedema (gas gangrene) in man and animals.	Wound
	② Malignant oedema (gangrenous dermatitis) in chickens	Wound
	③ Enterotoxaemia in sheep and calves	ingestion
	④ Food poisoning in man	ingestion
B	1- Lamb dysentery 2- enterotoxaemia in calves	Ingestion
C	1- struck disease in adult sheep 2- enterotoxaemia in calves 3- Necrotic enteritis in chickens	ingestion
D	1- pulpy kidney in young sheep 2- enterotoxaemia in calves	ingestion
E	1- Necrotic enteritis in man 2- enterotoxaemia	ingestion
F	Enterotoxaemia	ingestion

● diseases caused by C. perfringens type B and C are due to consumption of excessive amounts of Food Low in protein and rich in CHO → because it is accompanied by low production of pancreatic trypsin or other proteases which destroy B-toxin → so, allowing B-toxin to act in the small intestine.

● Sudden death occurs within 24 hrs due to toxaemia.

## C. Septicum

diseases	Route
1- Malignant oedema in man and animals	Wound
2- gangrenous dermatitis in chicken	Wound
3- Braxy (Bradsot) in sheep.	ingestion

## C. chauvoei

diseases	Route
1- Symptomatic anthrax in sheep.	Wound
2- Black quarter (Black Leg) in cattle and buffaloes	ingestion

# C. novyi

Type	diseases	susceptible animals	Route
A	① Malignant oedema ② gangrenous dermatitis ③ Swelled head in breeding rams	Man and animals chickens  Ram	Wound
B	1- Black disease (Infectious necrotic hepatitis) → Common in animals suffer from Liver Fluke infestation. 2- Swelled head in breeding rams	Sheep and Cattle  Ram	ingestion  Wound
C	Non-toxigenic		
D (C. haemolyticum)	Red Water disease (Infectious ictero-haemoglobinuria or Bacillary haemoglobinuria)	Cattle and sheep	ingestion

## ● Malignant oedema (gas-gangrene)

In man and animals:

→ Caused by

- C. perfringens type A
- C. septicum
- C. novyi type A
- C. Sordelli

→ In chicken, It is called gangrenous dermatitis and

Caused by

- C. perfringens type A
- C. septicum
- C. novyi type A
- C. Sporogenes

With or without staphylococci

## ● ulcerative enteritis (Quail disease):

Caused by C. Colinum

## ● omphalitis (Mushy chick disease):

Caused by clostridia with other bacteria (E. coli)

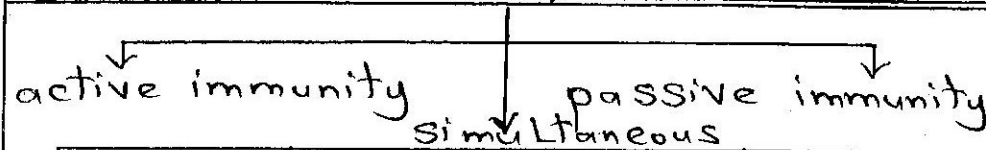


# Immunity

## I Natural (Innate) immunity:

- dogs, cats and fowls are naturally resistant to tetanus.
- Man, equines, dogs and fowls are naturally resistant to *C. chauvoei* infection

## II Acquired (adaptive) immunity:



### 1 - active Immunity

by vaccination

#### a) Vaccines used for clostridia:

1 - aluminium phosphate precipitated toxoid: deep I/M

→ used for *C. tetani* and *C. novyi*

2 - alum precipitated formalized whole culture vaccine:

→ It contains inactive organisms and toxoids → for *C. perfringens* type A, *C. septicum* and *C. chauvoei*  
 → deep I/M

#### 3 - Formalized whole culture vaccine:

→ *C. perfringens* type B and D and *C. novyi*  
 → S/C

#### 4 - polyvalent vaccine for malignant oedema (gas gangrene):

It is prepared from *C. perfringens* type A, *C. septicum* and *C. novyi* type A.

#### 5 - Co-vaccine (collective vaccine for most or all anaerobic diseases):

→ It is prepared from 8 strains  
 → given S/C

#### N.B:

#### Preparation of tetanus toxoid:

by addition of 0.3% formalin to culture filtrate → incubated for several weeks → then, the toxoid is purified by precipitation with aluminium phosphate.

## **(b) Vaccination against Lamb dysentery and pulpy Kidney:**

(C. perfringens type B and D)

→ as the infection occurs early in Lambs from few hrs of Life (Lamb dysentery) or from 3 months of Life (pulpy Kidney) → induction of immunity depends on natural passive immunity from the mother to the newly born Lambs through Colostrum.

### **1-Vaccination of pregnant ewe:**

2 doses of formalized whole culture of C. perfringens type B and D.

1st dose: 5 wks before parturition (S/C)

2nd dose: 2 wks after 1st dose (the vaccine is active for mother and passive for Lamb)

→ after parturition, the Lamb must receive Colostrum from the immunized dams → because it contains high conc. of B and E antitoxins (natural passive immunity)

### **2-Vaccination of Lambs:**

3 months old Lambs are vaccinated by formalized C. perfringens type B and D vaccine →

3 successive injections with one month interval (active vaccination of Lambs).

## **2-passive immunity**

- used only for C. tetani
- by using antitetanic serum (antitoxin) which gives immediate protection and remain for one month.
- used for

### prophylactic measures

2 mL (1500 IU/mL) are injected IM immediately post-accidents or post-operations or in severe burns.

### therapeutic measures

Single Large dose of 10-20 mL is injected I/M in human and in highly expensive race horses.

- Repeated dose of anti-tetanic serum may cause serum sickness

## **3-Simultaneous**

(active and passive immunity in the same animal)

- applied by injection of tetanus toxoid on one side of neck and antitetanic serum on the other side.

- It gives immediate protection as well as Long Lasting immunity at the same time.